JOURNAL OF CLINICAL MICROBIOLOGY, July 2003, p. 3368–3371 Vol. 41, No. 7
0095-1137/03/$08.00+0 DOI: 10.1128/JCM.41.7.3368–3371.2003

Tsukamurella Conjunctivitis: a Novel Clinical Syndrome

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Received 17 March 2003/Returned for modification 13 April 2003/Accepted 19 April 2003

In this report, we describe the first three cases of *Tsukamurella* conjunctivitis in the literature. All three patients presented with congestion of one eye with small amounts of serous discharge for 1 to 2 days. All three recovered after 10 days of treatment with polymyxin B-neomycin or chloramphenicol eyedrops. Sequencing of the 16S rRNA genes of the three isolates recovered from the serous discharge of the three patients showed that they were all *Tsukamurella* species. The phenotypic characteristics of the isolate obtained from one patient best fit the phenotypic profile of *Tsukamurella pulmonis*, whereas those of the other two best fit that of *Tsukamurella tyrosinosolvens*.

Using the information obtained from analysis of 16S rRNA gene sequences, *Tsukamurella* was first proposed as a genus in 1988 (2), although the first strain of this group of bacteria was first described in 1941 (19), and the first isolate from humans was reported in 1971 (21). Similar to related genera of the order *Actinomycetales*, such as *Nocardia*, *Rhodococcus*, and *Gordonia*, members of *Tsukamurella* are gram positive, aerobic, catalase positive, and partially acid-fast as a result of the presence of mycolic acid in the cell envelope. Due to their similar phenotypic properties, differentiation of and speciation within these genera are difficult in most clinical microbiology laboratories.

Among the seven known species of *Tsukamurella*, *T. inchonensis*, *T. paurometabola*, *T. pulmonis*, *T. strandjordii*, and *T. tyrosinosolvens* have been reported to cause infections in humans (1, 4–6, 8, 10, 12, 14, 15, 17, 28–30). The most common *Tsukamurella* infections in human are indwelling device-related infections, including catheter-related bacteremia, especially that of the central venous catheter (7, 10, 14, 16, 17), peritonitis associated with continuous ambulatory peritoneal dialysis (15), and knee prosthesis infection (8). Most cases of human infection reported in the literature were caused by *T. paurometabola*. In this article, we describe the first three cases of *Tsukamurella* conjunctivitis in the literature. The three *Tsukamurella* species were identified by a combination of phenotypic tests and 16S rRNA gene sequencing. The importance of a continuous search for novel clinical syndromes is also discussed.

All clinical data were collected prospectively. Clinical specimens were collected and handled according to standard protocols (11). All suspect colonies were identified by standard conventional biochemical methods (11) and with the API 20C AUX and API 50 CH systems (bioMerieux, Lyon, France), using *T. paurometabolum* (ATCC 8363) and *T. pulmonis* (ATCC 700081) as controls. Antibiotic susceptibility testing was performed by the broth macrodilution method (11). Bacterial DNA extraction and 16S rRNA gene sequencing were performed as described in previous publications (9, 18, 22), with LPW57 (5′-AGTTTGATCCTGGCTCAG-3′) and LPW58 (5′-AGGCCCGGGAACGTATTCAC-3′) (Gibco BRL, Rockville, Md.) as the PCR and sequencing primers. The sequences of the PCR products were compared with known 16S rRNA gene sequences in GenBank by multiple sequence alignment with the CLUSTAL W program (20), and phylogenetic tree construction was performed by PileUp and the neighbor-joining method with GrowTree (Genetics Computer Group, Inc.).

Case 1. A 38-year-old Chinese man with a history of good health presented with left eye congestion and serous discharge for 1 day in January 2001. There was no pain, photophobia, blurring of vision, or fever, and there was no history of injury, wearing contact lens, or swimming within 2 weeks prior to onset of symptoms. He recovered after 10 days of treatment with chloramphenicol eyedrops.

Case 2. A 69-year-old Chinese woman with hypertension and bronchogenic carcinoma with right upper lobectomy presented with left eye congestion and serous discharge for 2 days in April 2002. There was no pain, photophobia, blurring of vision, or fever, and there was no history of injury, wearing contact lens, or swimming within 2 weeks prior to onset of symptoms. The husband of the patient also had a similar illness at the time that she developed conjunctivitis. She recovered after 10 days of treatment with polymyxin B-neomycin eyedrops.

Case 3. A 27-year-old Chinese man with a history of good health presented with congestion, serous discharge, and itchiness of the left eye for 2 days in May 2002. There was no pain, photophobia, blurring of vision, or fever, and there was no history of injury, wearing contact lens, or swimming within 2 weeks prior to the onset of symptoms. He recovered after 10 days of treatment with chloramphenicol eyedrops.

Gram smear of the serous discharge of all three patients showed copious amounts of polymorphs. On day 2 postincubation, a pure culture of gram-positive aerobic nonsporulating bacillus was recovered from cultures of the discharge from all three patients. All three isolates were acid-fast according to a modified acid-fast stain. All three isolates grew on blood agar as yellow, rough, irregular, and dry but easily emulsified colo-

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TABLE 1. Phenotypic characteristics of the three isolates in this study compared to those of other *Tsukamurella* species[a]

Phenotypic characteristic	Clinical isolate			*Tsukamurella* species[b]				
	1	2	3	*T. inchonensis*	*T. paurometabola*	*T. pulmonis*	*T. strandjordii*	*T. tyrosinosolvens*
Growth at 42°C	−	−	−	+	−	−	−	−
Hydrolysis of:								
Tyrosine	+	−	+	−	−	−	−	+
Xanthine	−	−	−	−	−	−	−	V
Assimilation of:								
Maltose	+	−	+	+	−	−	V	+
Cellobiose	−	−	−	+	−	−	NA	−
D-Melezitose	+	−	+	+	−	−	V	+
D-Sorbitol	+	+	+	+	−	+	NA	+
Glycerol	−	−	−	V	+	V	V	V
2-Ketogluconate	+	−	+	V	−	−	V	+
Xylitol	+	+	+	V	−	V	+	V
Inositol	+	−	+	+	−	−	+	+
α-Methyl-D-glucoside	+	−	+	+	−	−	V	V
D-Arabinose	−	−	−	V	−	V	−	V
Ribose	−	−	−	V	−	−	−	V
D-Mannose	−	+	−	+	−	+	+	V
Mannitol	+	+	+	+	−	+	+	+
Arbutine	+	−	−	V	−	−	+	V
Salicin	−	−	−	V	−	−	+	V
Inulin	+	−	+	+	−	−	+	+
L-Fucose	+	+	+	V	−	+	+	+
D-Arabitol	+	+	+	+	−	+	+	+

[a] +, present; −, absent; V, variable; NA, not available.
[b] For details, see references 6 and 28 to 30.

nies 2 mm in diameter after 48 h of incubation at 37°C in an aerobic environment with 5% CO_2. The major phenotypic characteristics of the three isolates for comparison with those of the other *Tsukamurella* species are summarized in Table 1. The phenotypic characteristics of the isolate obtained from patient 2 best fit the phenotypic profile of *T. pulmonis*, whereas those of the isolates obtained from patients 1 and 3 best fit that of *T. tyrosinosolvens*. No other bacterium or virus was recovered from the serous discharge.

The MICs of neomycin for isolates 1, 2, and 3 were <0.12 μg/ml, and those of chloramphenicol for isolates 1, 2, and 3 were 8, 2, and 4 μg/ml, respectively.

PCR of the 16S rRNA genes of the three eye pus isolates showed bands at about 1,300 bp. Sequencing of the 16S rRNA genes of the isolates showed that there were similarities of >99% between the 16S rRNA gene sequences of the isolates and those of other *Tsukamurella* species, indicating that the isolates were all *Tsukamurella* species (Fig. 1).

Conjunctivitis, or inflammation of the conjunctiva, is the most common type of ocular inflammation. Infections, allergens, and other irritative substances are the major causes of conjunctivitis, and infective conjunctivitis can be caused by bacteria, viruses, fungi, or parasites. In immunocompetent adults, the most common bacteria that cause acute conjunctivitis, defined by onset of symptoms within 3 weeks of presentation, are *Streptococcus pneumoniae* and *Staphylococcus aureus*. Less commonly, the following species have also been reported to cause acute conjunctivitis: *Streptococcus epidermidis*, *Streptococcus pyogenes*, and viridans streptococci; *Haemophilus influenzae* and *Haemophilus ducreyi*; *Neisseiria gonorrhoeae* and *Neisseria meningitidis*; *Moraxella lacunata* and *Moraxella catarrhalis*; *Proteus vulgaris*; *Corynebacterium diphtheriae*; *Shigella flexneri*; *Yersinia enterocolitica*; *Acinetobacter*; and *Aeromonas hydrophila.* In this article, with the help of both phenotypic tests and 16S rRNA gene sequencing, we defined a series of three cases of community-acquired acute conjunctivitis associated with *Tsukamurella*, a bacterial genus that had never previously been reported to cause conjunctivitis.

The clinical features of *Tsukamurella* conjunctivitis are those of acute conjunctivitis of mild severity. The most common clinical features of acute conjunctivitis are hyperemia due to dilatation and congestion of the blood vessels and the presence of secretions, the type of which depends on the etiology of the disease. In mild conjunctivitis, apart from mild hyperemia and scanty discharge, there is usually no edema of the eyelid or corneal involvement (characterized by photophobia, visual impairment, a gritty sensation, and pain). These characteristics of mild conjunctivitis were present in the three patients in the present series, all of whom had unilateral conjunctival hyperemia and serous discharge present for 1 to 2 days before presentation. All three patients responded promptly to eyedrops containing polymyxin B-neomycin or chloramphenicol, which are commonly used for the treatment of conjunctivitis due to gram-positive bacteria.

16S rRNA gene sequencing can be used for identification of *Tsukamurella* to the genus level, but is not discriminative enough for speciation within this genus. Despite the success in using 16S rRNA gene sequencing for identifying most bacterial species, there are "blind spots" within some major genera, in which 16S rRNA gene sequences have been found to be not discriminative enough for the identification of certain species. In such circumstances, sequences of essential genes other than

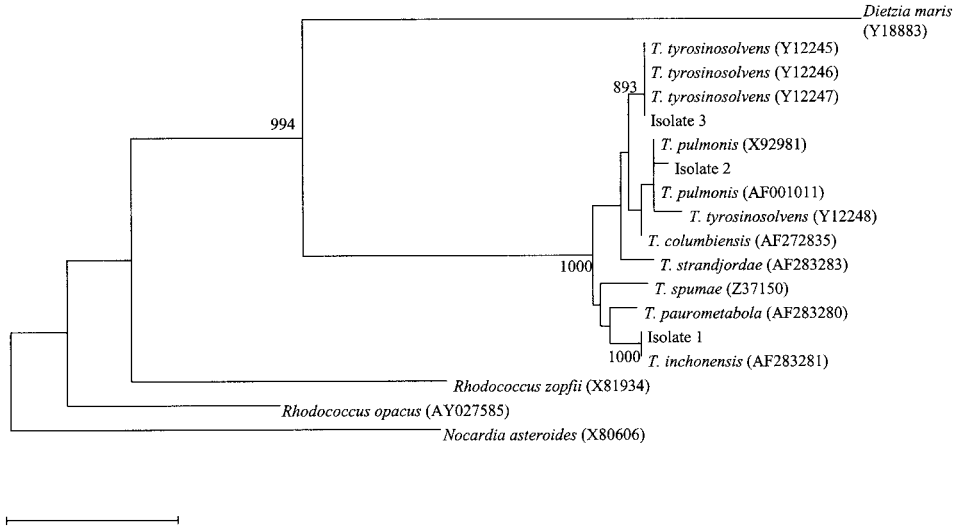

FIG. 1. Phylogenetic tree showing the relationship of the three isolates from our patients to related species. The tree was inferred from 16S rRNA sequence data by the neighbor-joining method and bootstrap values calculated from 1,000 trees. The scale bar indicates the estimated number of substitutions per 100 bases with the Jukes-Cantor correction. Names and accession numbers are given as cited in the GenBank database.

16S rRNA, such as *groEL*, have been shown to be useful for the identification of some species that cannot be discriminated by 16S rRNA gene sequencing (3, 13). Recently, we have used *groEL* gene sequencing to distinguish between *Burkholderia pseudomallei* and *Burkholderia thailandensis* (24). The difference between the 16S rRNA gene sequences of the two species is just 1%, but the difference between the *groEL* gene sequences of the two species is >2.4% and hence is a better target than the 16S rRNA gene for discrimination between the two species. Interestingly, we have also found that 16S rRNA gene sequencing is not good for discrimination with the genus *Microbacterium*, another genus of gram-positive aerobic rod similar in microscopic appearance to *Tsukamurella* (9). In this study, 16S rRNA gene sequencing confirmed that the three isolates were *Tsukamurella*. However, the differences among the 16S rRNA gene sequences of the various *Tsukamurella* species are small. Therefore phenotypic tests were used for speciation of the three isolates. In the GenBank database, the *groEL* gene sequences of only *T. tyrosinosolvens* (GenBank accession no. U90204) and *T. paurometabola* (GenBank accession no. AF352578) are available. There is 5.4% difference between the *groEL* gene sequences of the two strains, as opposed to <1% difference between the 16S rRNA gene sequence of *T. tyrosinosolvens* (GenBank accession no. Y12245) and that of *T. paurometabola* (GenBank accession no. AF283280). Therefore, the *groEL* gene is a potentially good target for *Tsukamurella* speciation. Further studies on *groEL* gene sequencing of multiple strains of each species of *Tsukamurella* should be performed so as to ascertain whether this gene target is good for the discrimination of the different species of *Tsukamurella*.

A continuous search for novel clinical syndromes, either in the form of previously undescribed pathogens or in the form of known microbes causing clinical syndromes that were not known to be caused by the corresponding microbes (as in the present report on *Tsukamurella* conjunctivitis), is important. Two years ago, we discovered that about one-fourth of negative blood cultures that were believed to be of infective origin in bone marrow transplant recipients with fever and neutropenia were due to bacteremia caused by cell-wall-deficient forms of bacteria (27). Last year, we also demonstrated that rapidly growing mycobacteria can cause acupuncture-transmitted skin and soft tissue infections (25, 26). Furthermore, novel bacteria that are potentially important pathogens are continuously being discovered (23, 31). These discoveries not only help us to have a better understanding of the biological basis behind the corresponding infections, but also help us towards better laboratory diagnosis as well as empirical and definitive treatment of the various infections.

Nucleotide sequence accession number. The 16S rRNA gene sequences of the three isolates recovered from the three patients in this study have been submitted to the GenBank sequence database under accession no. AY254698, AY254699, and AY253916, respectively.

This work was partly supported by the University Development Fund, University Research Grant Council, and the Committee for Research and Conference Grant, The University of Hong Kong.

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